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# Pharmacological Profile of the Novel Antidepressant 4-(2-Fluorophenyl)-6-methyl-2-(1-piperazinyl)thieno-[2,3-d]pyrimidine Monohydrate Hydrochloride

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## Summary

This is a first report on the investigation of the antidepressant activity of MCI-225 (4-(2-fluorophenyl)-6-methyl-2-(1-piperazinyl)thieno[2,3-d]pyrimidine monohydrate hydrochloride, CAS 99487-26-0) in comparison with maprotiline (CAS 10347-81-6), desipramine (CAS 58-28-6), imipramine (CAS 113-52-0) and trazodone (CAS 25332-39-2). MCI-225 inhibited the synaptosomal uptake of noradrenaline (NA, $K_i$ = 35.0 nmol/l), serotonin (5-HT, $K_i$ = 491 nmol/l), and dopamine ($K_i$ = 14800 nmol/l), although it did not inhibit MAO-A and MAO-B activities. MCI-225 showed high affinity only for the 5-$HT_3$ receptor ($K_i$ = 81.0 nmol/l) among all receptors tested including $M_1$, $M_2$, $\alpha_1$, and $H_1$ receptors. The inhibition of the von Bezold-Jarisch reflex by MCI-225 ($ID_{50}$ = 22.2 mg/kg, p.o.) suggests its antagonistic action on the 5-$HT_3$ receptor. MCI-225 dose-dependently reduced reserpine-induced hypothermia (0.3–10 mg/kg, p.o.) and potentiated yohimbine-induced lethality (3–100 mg/kg, p.o.) in mice. These effects of MCI-225 were as potent as desipramine and more potent than maprotiline, imipramine and trazodone. MCI-225 and desipramine did not change either 5-HTP-induced head movements or p-CA-induced hyperactivity in rats. In forced swimming tests in rats, the minimum effective doses of MCI-225, maprotiline, desipramine, and imipramine were 1, 30, 10 and 30 mg/kg, p.o., respectively, for 5-days administration. Only MCI-225 had shown its full activity with this short term treatment. MCI-225 (10 mg/kg, p.o.) decreased the REM sleep period without affecting slow-wave sleep or wakefulness in rats. Even at 100 mg/kg, p.o. MCI-225 and trazodone did not inhibit oxotremorine-induced tremor, lacrimation or salivation in mice in contrast with imipramine. These results suggest that MCI-225, which selectively inhibits NA uptake and antagonizes the 5-$HT_3$ receptor, has potential as a new type of potent antidepressant.

## Zusammenfassung

*Pharmakologisches Profil des neuen Antidepressivums 4-(2-Fluorphenyl)-6-methyl-2-(1-piperazinyl)-thieno[2,3-d]pyrimidin-monohydrat-hydrochlorid*

Dies ist der erste Bericht über die Erforschung der antidepressiven Wirkung von MCI-225 (4-(2-Fluorphenyl)-6-methyl-2-(1-piperazinyl)thieno[2,3-d]pyrimidin-monohydrat-hypochlorid, CAS 99487-26-0) im Vergleich mit Maprotilin (CAS 10347-81-6), Desipramin (CAS 58-28-6), Imipramin (CAS 113-52-0) und Trazodon (CAS 25332-39-2). MCI-225 hemmte die synaptosomale Aufnahme von Noradrenalin ($K_i$ = 35,0 nmol/l), Serotonin ($K_i$ = 491 nmol/l) und Dopamin ($K_i$ = 14800 nmol/l), während es die Aktivitäten von MAO-A und MAO-B nicht hemmte. MCI-225 zeigte nur für den 5-$HT_3$-Rezeptor eine hohe Affinität ($K_i$ = 81,0 nmol/l) unter allen getesteten Rezeptoren einschließlich $M_1$, $M_2$, $\alpha_1$ und $H_1$.

Die Hemmung des von-Bezold-Jarisch-Reflexes durch MCI-225 ($ID_{50}$ = 22,2 mg/kg p.o.) legt seine antagonistische Wirkung auf den 5-$HT_3$-Rezeptor nahe. MCI-225 reduzierte dosisabhängig die Reserpin-induzierte Hyperthermie (0,3–10 mg/kg p.o.) und potenzierte die Yohimbin-induzierte Sterblichkeit (3–100 mg/kg p.o.) bei Mäusen. Diese Wirkungen von MCI-225 waren so stark wie diejenigen von Desipramin und stärker als diejenigen von Maprotilin, Imipramin und Trazodon. MCI-225 und Desipramin hatten keinen Einfluß auf durch 5-HTP ausgelöste Kopfbewegungen oder durch p-CA ausgelöste Überaktivität bei Ratten. Im Schwimmtest bei Ratten waren die minimalen effektiven Dosen für MCI-225, Maprotilin, Desipramin und Imipramin 1, 30, 10 bzw. 30 mg/kg p.o. bei Stägiger Gabe. Nur MCI-225 zeigte während dieser kurzfristigen Behandlung volle Wirksamkeit. MCI-225 (10 mg/kg p.o.) verringerte die REM-Schlaf-Periode, ohne den durch langsame Wellen gekennzeichneten Schlaf oder die Wachheit zu beeinträchtigen.

Selbst bei 100 mg/kg p.o. hemmten MCI-225 und Trazodon im Gegensatz zu Imipramin den Oxotremorin-induzierten Tremor, Tränen- und Speichelfluß nicht. Diese Ergebnisse legen nahe, daß MCI-225, das die Noradrenalin-Aufnahme selektiv hemmt und 5-$HT_2$-Rezeptoren antagonisiert, Potential als ein starkes Antidepressivum neuen Typs hat.

**Key words** Antidepressants · CAS 99487-26-0 · 4-(2-Fluorophenyl)-6-methyl-2-(1-piperazinyl)thieno[2,3-d]pyrimidine monohydrate hydrochloride · MCI-225, antidepressant activity, 5-$HT_2$ receptor antagonism, noradrenaline uptake inhibition, pharmacology

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## 1. Introduction

Ever since the discovery of imipramine, a monoamine uptake inhibitor, in the early 1950s a number of antidepressants have been developed. Most of these antidepressants interact with the noradrenergic and/or serotonergic systems in the central nervous system and show clinical effectiveness in the treatment of depression [1]; noradrenaline (NA) and serotonin (5-HT) are believed to play an important and multifunctional role in depression [2]. Among these antidepressants, the traditional agents, such as tricyclic antidepressants (TCA) show potent efficacy but they also have adverse effects which limit their use, such as anti-cholinergic side effects, alteration of cardiac condition or sedation [3]. Newer agents, selective serotonin reuptake inhibitors (SSRI) have become popular due to their fewer anti-cholinergic, sedative, psychomotor, and cardiovascular side effects [4, 5], but some investigators do not find SSRI medications to be as effective for severe depression as TCAs [6]. Therefore, newer antidepressants are still needed and many new antidepressants candidates are being investigated.

MCI-225 (4-(2-fluorophenyl)-6-methyl-2-(1-piperazinyl) thieno[2,3-d]pyrimidine monohydrate hydrochloride, CAS 99487-26-0), is a novel psychoactive compound that has been reported to improve amnesia in scopolamine-treated rats and basal forebrain-lesioned rats [7, 8]. Furthermore, MCI-225 reduced resistance to extinction of the food-rewarded runway response in dorsal noradrenergic bundle-lesioned rats, and improved the reduction in the number of approaches to a novel object in DSP-4, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine-treated mongolian gerbils [9]. In these models, the concentration of noradrenaline (NA) in the brain was markedly decreased and the function of the central noradrenergic neurons was thought to be impaired. This suggests that MCI-225 enhances the central noradrenergic function, at least in these models, although its mechanism of action remains unknown. For this paper, we investigated the neuropharmacological profiles of MCI-225 and evaluated its antidepressant activity in comparison with maprotiline (CAS 10347-81-6), desipramine (CAS 58-28-6), imipramine (CAS 113-52-0), and trazodone (CAS 25332-39-2).

## 2. Material and methods

### 2.1. Animals

Male Wistar rats and male Wistar-Imamichi rats (Japan Laboratory Animals, Inc., Tokyo, Japan, and Institute for animal reproduction, Ibaraki, Japan, respectively), weighing 150–400 g, and male ddY mice (Japan SLC, Inc. Shizuoka, Japan), weighing 15–40 g, were used. All animals were housed in groups kept in an air-conditioned room on a 12-h light-dark cycle (light period: 7:00–19:00) and given food and water ad libitum. Animals were sub-divided into groups for different drug treatment at randomized manner. All experiments were done between 9:00 and 18:00. Each animal was used only once. Details of the receptor binding study are shown in Table 1.

### 2.2. Drugs

MCI-225 and ondansetron monohydrochloride dihydrate (CAS 103639-04-9) were synthesized and maprotiline hydrochloride was extracted from a commercial preparation in our laboratory. Other compounds were purchased commercially. MCI-225, L-3,4-dihydroxyphenylalanine (L-DOPA, Sigma, St. Louis, MO, USA), and nialamide (Sigma) were suspended in 0.5 % Tween 80, and maprotiline was suspended in distilled water. Desipramine hydrochloride (Sigma), imipramine hydrochloride (Sigma), trazodone hydrochloride (Sigma), yohimbine hydrochloride (Sigma), and ondansetron were dissolved in distilled water. 5-Hydroxy-L-tryptophan (5-HTP, Sigma), methamphetamine hydrochloride (Dainihon, Oasaka, Japan), DL-p-chloroamphetamine hydrochloride (p-CA, Sigma), and serotonin hydrochloride (Research Biochemicals International, Natick, MA, USA) were dissolved in saline. Tetrabenazine (Fluka, Buchs, Switzerland) was dissolved in 0.1 N HCl and the solution was diluted to the appropriate concentration with distilled water. Reserpine (Apoplon, Daiichi, Tokyo, Japan) was also used. All drugs were prepared immediately before use and given in a volume of 1 ml/kg to rats or 10 ml/kg to mice. Animals in the control group were given the vehicle.

[$^3$H]AF-DX384(N-[2-[2[(dipropylamino)methyl]-1-piperidinyl] ethyl]-5,6-dihydro-6-oxo-11H-pyrido[2,3b][1,4]benzodiazepine-11-carboxamide, monomethanesulfonate), [$^3$H]p-aminoclonidine, [$^3$H]citalopram, [$^3$H]dopamine (DA), [$^3$H]DAMGO ([D-Ala$^2$,MePhe$^4$,Gly-ol$^5$]enkephalin), [$^3$H]dexamethasone, [$^3$H]dihydroalprenolol, [$^3$H]DPDPE ([D-Pen$^2$,D-Pen$^5$]enkephalin), [$^3$H]flunitrazepam, [$^3$H]GR65630(3-(5-methyl-1H-imidazol-4-yl)-1-(1-methyl-1H-indol-3-yl)-1propanone), [$^{14}$C]5-HT, [$^3$H]5-HT, [$^3$H]ketanserine, [$^3$H]LSD(lysergic acid diethylamaide), [$^3$H]N-methylcarbamyl choline iodide, [$^3$H]naloxone, [$^3$H]NA, [$^3$H]nisoxetine, [$^3$H]8-OH-DPAT(8-hydroxy-2(di-n-propylamino) tetralin), [$^{14}$C]phenylethylamine, [$^3$H]pirenzepine, [$^3$H]prazosin, [$^3$H]pyrilamine, [$^3$H]quinuclidinyl benzilate, [$^3$H]SCH23390((R)-(+)-8-chloro-2,3,4,5,-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol), [$^3$H]scopolamine, [$^3$H]sulpiride, [$^{125}$I]tyrO-oCRF (corticotropin releasing factor), and [$^3$H]tiotidine were obtained from Du Pont / NEN Research Products (Boston, MA, USA). [$^3$H]GABA (γ-aminobutyric acid), [$^3$H]GR113808([1-[2-[(methylsulfonyl)amino]ethyl-4-piperdinyl]methyl-1-methyl-1H-indole-3-carboxylate), [$^3$H]mesulergine and [$^3$H]U-69593 ([5R-(5α,7α,8β)]-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro [4,5] dec-8-yl]-benzeneacetamide were from Amersham International (Arlington Heights, IL, USA).

### 2.3. Synaptosomal uptake of $^3$H-NA, 5-HT and DA

Rats were decapitated and the cortical, hypothalamic, hyppocampal and striatal tissues were rapidly dissected. The tissues were homogenized (Potter homogenizer with Teflon pestle) in 10 volumes of ice cold 0.32 mol/l sucrose. The $P_2$ fraction was obtained by centrifugation at 1000 × g for 10 min and 11500 × g for 20 min and suspended in Krebs-Ringer phosphate buffer, pH 7.4 (124 mmol/l NaCl, 5 mmol/l KCl, 20 mmol/l $Na_2HPO_4$, 1.2 mmol/l $KH_2PO_4$, 1.3 mmol/l $MgSO_4$, 0.75 mmol/l $CaCl_2$, 10 mmol/l glucose). The [$^3$H]NA uptake experiments were performed on the cortical and hypothalamic synaptosome, [$^3$H] 5-HT uptake experiments were performed on the cortical, hypothalamic and hippocampal synaptosome, and [$^3$H]DA uptake experiments were performed on striatal synaptosome. The assay tubes contained radiolabeled ligands in a volume of 0.2 ml, compounds at 5 or more concentrations in a volume of 0.1 ml, and the oxygenated buffer described above in a volume of 0.5 ml. After 5 min preincubation at 37 °C, uptake was initiated by the addition of the synaptosomal fraction in a volume of 0.2

*Table 1:* Experimental details of each binding assay.

| Binding site | Radiolabeled ligand | Conc. (nmol/l) | Tissue | Incubation | | | Drug used to define non-specific binding | Conc. (μmol/l) | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Time (min) | Temp. (°C) | Buffer | | | |
| $\alpha_1$ | [$^3$H]Prazosin | 0.2 | rat cerebral cortex | 40 | 25 | A | Prazosin | 10 | 41 |
| $\alpha_2$ | [$^3$H]Aminoclonidine | 0.8 | rat cerebral cortex | 120 | 25 | A | Aminoclonidine | 10 | 42 |
| $\beta_1$ | [$^{125}$I]Iodopindilol | 0.2 | rat cerebral cortex | 60 | 37 | B[a] | Alprenolol | 10 | 43 |
| $\beta_2$ | [$^{125}$I]-Iodopindilol | 0.2 | rat cerebral cortex | 60 | 37 | B[b] | Alprenolol | 10 | 43 |
| 5-HT$_1$ | [$^3$H]5-HT | 2 | rat striatum | 45 | 25 | A | 5-HT | 10 | 44 |
| 5-HT$_{1A}$ | [$^3$H]8-OH-DPAT | 0.5 | rat cerebral cortex | 30 | 25 | D | 8-OH-DPAT | 10 | 44 |
| 5-HT$_{1c}$ | [$^3$H]Mesulergine | 0.7 | rat cerebral cortex | 30 | 37 | E | Mianserin | 10 | 44 |
| 5-HT$_2$ | [$^3$H]Ketanserine | 0.7 | rat cerebral cortex | 30 | 37 | A | 5-HT | 1000 | 45 |
| 5-HT$_3$ | [$^3$H]GR65630 | 0.3 | rat cerebral cortex | 30 | 37 | K | Metoclopramide | 300 | 46 |
| 5-HT$_4$ | [$^3$H]GR113808 | 0.2 | guinea pig striatum | 30 | 37 | K | 5-HT | 30 | 47 |
| 5-HT$_6$ | [$^3$H]LSD | 2 | rat recombinant SF9 cell | 60 | 37 | C | Methiothepin | 0.1 | 48 |
| 5-HT$_7$ | [$^3$H]LSD | 2 | rat recombinant SF9 cell | 60 | 37 | C | 5-CT | 10 | 49 |
| $D_1$ | [$^3$H]SCH23390 | 0.5 | rat striatum | 60 | 25 | F | SCH23390 | 0.1 | 50 |
| $D_2$ | [$^3$H]Sulpiride | 2 | rat striatum | 60 | 25 | G | Sulpiride | 10 | 51 |
| Muscarinic | [$^3$H]QNB | 0.2 | rat cerebral cortex | 60 | 37 | A | Atropine | 100 | 52 |
| $M_1$ | [$^3$H]Pirenzepine | 1 | bovine striatum | 60 | 25 | H | Atropine | 10 | 53 |
| $M_2$ | [$^3$H]AF-DX384 | 5 | rat heart | 30 | 25 | H | Methoctramine | 100 | 54 |
| $M_3$ | [$^3$H]Scopolamine | 1 | guinea pig ileum | 120 | 37 | I | Atropine | 10 | 55 |
| Nicotinic | [$^3$H]NMCl | 5 | rat cerebral cortex | 60 | 4 | L | Nicotine | 2 | 56 |
| $H_1$ | [$^3$H]Pyrilamine | 1 | rat cerebellum | 60 | 25 | A | d-Chlorpheniramine | 10 | 57 |
| $H_2$ | [$^3$H]Tiotidine | 2 | guinea pig cerebral cortex | 30 | 25 | J | Histamine | 5000 | 58 |
| GABA-A | [$^3$H]GABA | 5 | bovine cerebellum | 15 | 4 | A | GABA | 1 | 59 |
| GABA-B | [$^3$H]GABA | 5 | rat cerebral cortex | 15 | 25 | M | Baclofen | 100 | 60 |
| BZP | [$^3$H]Flunitrazepam | 0.5 | bovine cerebral cortex | 45 | 4 | H | Ro15-1788 | 0.5 | 61 |
| Opiate non-select. | [$^3$H]Naloxone | 1 | rat forebrain | 60 | 25 | A | Naloxone | 1 | 62 |
| Opiate κ | [$^3$H]U-69593 | 0.75 | guinea pig cerebellum | 120 | 30 | K | U-69593 | 1 | 63 |
| Opiate μ | [$^3$H]DAMGO | 1 | rat forebrain | 90 | 25 | A | Naloxone | 1 | 64 |
| Opiate δ | [$^3$H]DPDPE | 1 | rat forebrain | 90 | 25 | A | Naloxone | 10 | 65 |
| CRF | [$^{125}$I]TyrO-oCRF | 0.1 | rat cerebral cortex | 120 | 25 | Q | TyrO-oCRF | 1 | 66 |
| Glucocorticoid | [$^3$H]Dexamethasone | 1 | rat liver | 30 | 4 | R | Dexamethasone | 100 | 67 |
| NA transporter | [$^3$H]Nisoxetine | 0.6 | rat forebrain | 25 | 30 | N | Desipramine | 1 | 68 |
| 5-HT transporter | [$^3$H]Citalopram | 0.7 | rat forebrain | 60 | 25 | O | Clomipramine | 10 | 69 |

Buffer: A = 50 mmol/l Tris-HCl, pH 7.4; B = 50 mmol/l Tris-HCl, 150 mmol/l NaCl, 2.5 mmol/l $MgCl_2$ and 0.5 mmol/l ascorbate, pH 7.5; C = 50 mmol/l Tris-HCl, 10 mmol/l $MgCl_2$ and 1 mmol/l EDTA, pH 7.4; D = 50 mmol/l Tris-HCl, 10 μmol/l pargyline, 0.5 mmol/l ascorbate and 4 mmol/l $CaCl_2$, pH 7.4; E = 50 mmol/l Tris-HCl, 10 μmol/l pargyline, 0.5 mmol/l ascorbate and 10 nmol/l spiperone, pH 7.4; F = 50 mmol/l Tris-HCl, 120 mmol/l NaCl, 5 mmol/l KCl, 2 mmol/l $CaCl_2$ and 1 mmol/l $MgCl_2$, pH 7.4; G = 50 mmol/l Tris-HCl and 100 mmol/l NaCl, pH 7.5; H = 10 mmol/l Na-$KPO_4$, pH 7.4; I = 30 mmol/l HEPES, 142 mmol/l NaCl, 5.6 mmol/l KCl, 2.2 mmol/l $CaCl_2$, 3.6 mmol/l $Na_2CO_3$, 1 mmol/l $MgCl_2$ and 5.6 mmol/l glucose, pH 7.4; J = 50 mmol/l $Na_2PO_4$, pH 7.4; K = 50 mmol/l HEPES, pH 7.4; L = 50 mmol/l Tris-HCl, 120 mmol/l NaCl 5 mmol/l KCl, 2 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$ and 3 μmol/l atoropine, pH 7.4; M = 50 mmol/l Tris-HCl and 2.5 mmol/l $CaCl_2$, pH 7.4; N = 50 mmol/l Tris-HCl, 300 mmol/l NaCl and 5 mmol/l KCl, pH 7.4; O = 50 mmol/l Tris-HCl, 120 mmol/l NaCl and 5 mmol/l KCl, pH 7.4; P = 20 mmol/l HEPES and 150 mmol/l NaCl, pH 7.4; Q = 50 mmol/l HEPES, 10 mmol/l $MgCl_2$, 2 mmol/l EGTA, 0.1 mmol/l bacitracin, 0.1 % BSA and 0.12 TIU/ml aprotinin, pH 7.0; R = 0.5 mmol/l Tris-HCl and 0.5 mmol/l DTT, pH 7.4.
[a] containing 0.1 μmol/l $\beta_2$ blocker (ICI-118551), [b] containing 0.1 μmol/l $\beta_1$ blocker (ICI-89406).
5-CT:5-carboxamidetryptamine

ml. The final concentrations of [$^3$H]NA and [$^3$H]DA in the incubation mixtures were 0.25 and 0.4 μmol/l, respectively. The final concentrations of [$^3$H]5-HT in the cortical, hypothalamic and hippocampal synaptosome incubation mixtures were 0.02, 0.04, and 0.08 μmol/l, respectively. The reaction was stopped after 5 min ([$^3$H]NA and [$^3$H]5-HT) or 3 min ([$^3$H]DA) by filtration through a Whatman GF/B glass fiber filter under a vacuum with a cell harvester. The filter was rinsed three times with 4 ml of saline and placed in a scintillation vial containing 10 ml of Atomlight (Du Pont / NEN Research Products). Radioactivity was measured by liquid scintillation spectrometry. For the determination of non-specific uptake incubations were performed at 4 °C without the addition of test compounds. $IC_{50}$ values were calculated by nonlinear regression analysis. Inhibitor constants, $K_i$ values, were calculated from the $IC_{50}$ values based on the Cheng-Prusoff [10] equation as follows:

$$K_i = IC_{50} / (1 + [L]/K_d)$$

where [L] is the concentration of the radioligand and $K_d$ is the equilibrium dissociation constant of the radioligand.

## 2.4. Inhibition of radioligand binding

Appropriate membranes were incubated under the conditions described in Table 1 and then filtered through a Whatman GF/B glass fiber filter under a vacuum with a cell harvester. Radioactivity was measured by liquid scintillation spectrometry. $IC_{50}$ values were calculated by nonlinear regression analysis. In the binding study for NA and 5-HT transporter, the affinity constants, $K_i$ values, for MCI-225 were also calculated based on the Cheng-Prusoff [10] equation. In the case of the 5-HT$_3$ receptor, the reactions were performed directly on N1E-115 mouse neuroblastoma cells in 20 mmol/l HEPES buffer (pH 7.4) containing 150 mmol/l NaCl, 0.35 mmol/l [$^3$H]GR65630, and the tested compound at 6 or more concentrations at 25 °C for 60 min [11]. The reaction was terminated by rapid vacuum filtration onto glass fiber filter. Radioactivity trapped on the filters was measured by scintillation spectrometry. Non-specific binding was determined using 1 μmol/l MDL-7222 (endo - 8 - methyl - 8 - azabicyclo [3.2.1] oct - 3 - yl - 3,5 - dichlorobenzoate). $IC_{50}$ values were calculated by nonlinear regression analysis. The affinity constants, $K_i$ values, were calculated from the $IC_{50}$ values based on the Cheng-Prusoff [10] equation.

## 2.5. Reserpine-induced hypothermia

Reserpine (5 mg/kg, i.p.) and test compounds (p.o.) were administered simultaneously to mice. Rectal temperature was measured 4 h after treatment.

## 2.6. Tetrabenazine-induced ptosis

Tetrabenazine (12.5 mg/kg, i.p.) was administered to rats 1 h after oral administration of test compounds. Ptosis was scored 0.5, 1, 2, 3, or 4 h after tetrabenazine administration according to the method of Janssen et al. [12] (0 = normal, 1 = ¼, 2 = ½, 3 = ¾, 4 = complete closure of the eyelid).

## 2.7. Yohimbine-induced lethality

One hour after the administration (p.o.) of test compounds, yohimbine (30 mg/kg i.p.) was injected into 8 mice. The number of surviving mice was counted 2 h later. The $LD_{50}$ value was calculated by Probit analysis.

### 2.8. 5-HTP-induced head movements

The method of Katoh et al. [13] was used with modification. In brief, 1 h after the oral administration of test compounds, 5-HTP (150 mg/kg, i.p.) was injected into rats. 30 min after 5-HTP administration, the number of head movements, including both head-shaking and head-weaving, was counted for 5 min.

### 2.9. p-CA-induced hyperactivity

After 1 h habituation to an activity cage (24 × 37 × 30 cm), a pair of rats were administered test compounds (p.o.) and 30 min later injected with p-CA (5 mg/kg, s.c.). Locomotor activity was measured with an electromagnetic activity meter (Animex auto, Muromachi Kikai, Tokyo, Japan) for 90 min.

### 2.10. Methamphetamine-induced stereotyped behavior

Rats were orally given test compounds 1 h before methamphetamine (3 mg/kg, s.c.) administration. Stereotyped behavior was scored 0.5, 1, 2, 3, 4 h after methamphetamine treatment according to the criteria of Tobe et al. [14] (0 = asleep, eyes closed, 1 = motionless, eyes open, 2 = moving around the cage, 3 = sniffing, 4 = continuous licking or gnawing).

### 2.11. L-DOPA-induced behavioral syndrome

Mice were pretreated with nialamide (60 mg/kg, p.o.) 19 h before the administration of test compounds (p.o.). L-DOPA (100 mg/kg, i.p.) was administered 1.5 h thereafter and L-DOPA-induced behavioral syndromes, mainly piloerection, salivation and excitation with increased motor activity, were scored 1 h after L-DOPA administration according to the method of Ueki et al. [15] (1 = slight, 2 = moderate with increased touch response, 3 = severe with increased touch response, biting and Straub tail).

### 2.12. Forced swimming test

The method of Porsolt et al. [16] was used with modification. Briefly, a rat underwent 15 min and 5 min forced swimming trials with a 24-h intertrial interval. At second trial, the duration of immobility was recorded. The effects of single or repeated (5 and 14 days) oral administration of test compounds were investigated. The final two administrations were given 1 h before the start of the forced swimming trials.

### 2.13. Spontaneous motor activity

One hour after the oral administration of test compounds, the locomotor activity of a rat was recorded using an electromagnetic activity meter (Animex auto, Muromachi Kikai) in an activity cage (24 × 37 × 30 cm) for 5 min. The effect of 14-days administration of MCI-225 on the locomotor activity of rats was also examined by the same method.

### 2.14. Rapid eye movement sleep

Under pentobarbital sodium anesthesia (Nembutal, Dainihon, 40 mg/kg, i.p.), rats were implanted with electrodes for the chronic recording of ECoGs, EMG, and EOG activities in the frontal and occipital cortex, dorsal neck musculature and ocular orbit. Approximately one week after surgery, the rats were habituated for three days to a recording condition, and ECoGs, EMG and EOG were recorded using a polygraph (Nihon Koden, Tokyo, Japan) for 6 h after the oral administration of test compounds. Rapid eye movement sleep (REMS), slow wave sleep (SWS) and wakefulness were distinguished based on ECoGs, EMG, and EOG characteristics.

### 2.15. Von Bezold-Jarisch reflex

Rats were anesthetized with urethane (12.5 mg/kg, i.p.) 1 h after oral administration of MCI-225 or ondansetron. The right femoral vein was cannulated with a polyethylene tube for 5-HT injection. ECG and heart rate were monitored by standard methods using a polygraph (NEC medical systems, Tokyo, Japan) and 1 h later 5-HT (20 μg/kg) was injected. The area under the curve, which describes the bradycardia-quantified effect, was measured. The $ID_{50}$ value was calculated from the regression line.

### 2.16. Oxotremorine-induced tremor, salivation and lacrimation

One hour after the oral administration of test compounds, mice were injected with oxotremorine (0.2 mg/kg, i.p.). 30 min later, tremor intensity was scored according to the method of Ogren et al. [17] as followed, and the incidences of salivation and lacrimation were observed. (0 = no tremor, 1 = moderate, discontinuous tremor, 2 = intense, continuous tremor involving the whole body).

### 2.17. Statistics

The significance of parametric data in the behavior tests was analyzed by Dunnett's test following ANOVA. The significance of non-parametric data in behavior tests was analyzed by non parametric Dunnett's test following Kruskal-Wallis test. Differences between groups were considered to be significant if $p < 0.05$.

## 3. Results

### 3.1. Synaptosomal uptake of $^3$H-NA, $^3$H-5-HT and $^3$H-DA

As shown in Fig. 1 and Table 2, MCI-225 inhibited the uptake of [$^3$H]NA in both cortical and hypothalamic synaptosomes as potently as maprotiline and imipramine, but not as much as desipramine. The inhibition of the

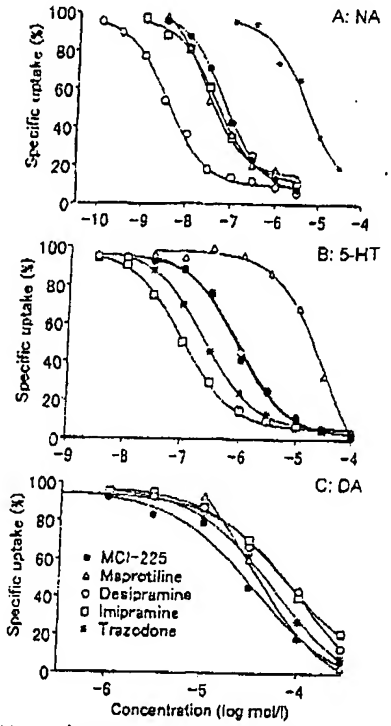


*Fig. 1:* Inhibition of [$^3$H]NA (*A*), [$^3$H]5-HT (*B*), and [$^3$H]DA (*C*) uptake into rat brain synaptosomes. The experiments with [$^3$H]NA and [$^3$H]5-HT were performed in hypothalamic synaptosomes. The experiment with [$^3$H]DA was performed in striatal synaptosome. Each point shows the result of an experiment performed in duplicate.

*Table 2:* Inhibition of [$^3$H]monoamine uptake by synaptosomes from rat brain tissues.

| Compound | Ki (nmol/l) | | | | | |
|---|---|---|---|---|---|---|
| | [$^3$H]NA | | [$^3$H]5-HT | | | [$^3$H]DA |
| | Cortex | Hypothalamus | Cortex | Hypothalamus | Hippocampus | Striatum |
| MCI-225 | 0.696 | 35.0 | 1070 | 491 | 244 | 14800 |
| Maprotiline | 1.15 | 19.7 | 4870 | 7900 | 5910 | 20800 |
| Desipramine | 0.227 | 1.81 | 1140 | 537 | 273 | 39400 |
| Imipramine | 4.50 | 22.2 | 194 | 56.3 | 24.1 | 35200 |
| Trazodone | 1160 | 2200 | 433 | 130 | 74.6 | 23900 |

*Table 3:* Inhibition of radiolabeled ligand binding by MCI-225. Experimental details are summarized in Table 1.

| Binding site | $IC_{50}$ (μmol/l) |
|---|---|
| $\alpha_1$ | > 10 |
| $\alpha_2$ | 5.8 |
| $\beta_1$ | > 10 |
| $\beta_2$ | > 10 |
| 5-$HT_1$ | > 10 |
| 5-$HT_{1A}$ | > 10 |
| 5-$HT_{1c}$ | > 10 |
| 5-$HT_2$ | > 10 |
| 5-$HT_3$ | 0.081 |
| 5-$HT_4$ | 4.2 |
| 5-$HT_6$ | 5.3 |
| 5-$HT_7$ | 2.5 |
| $D_1$ | > 10 |
| $D_2$ | 4.9 |
| Muscarinic | 9.1 |
| $M_1$ | 7.3 |
| $M_2$ | 3.7 |
| $M_3$ | > 10 |
| Nicotinic | > 10 |
| $H_1$ | 2.8 |
| $H_2$ | > 10 |
| GABA-A | > 10 |
| GABA-B | > 10 |
| BZP | > 10 |
| Opiate non-selective | > 10 |
| Opiate κ | 2.0 |
| Opiate μ | > 10 |
| Opiate δ | 5.0 |
| CRF | > 10 |
| Glucocorticoid | > 10 |
| NA transporter | 0.053 |
| 5-HT transporter | 0.36 |

uptake of [$^3$H]5-HT by MCI-225 was less potent than that with either imipramine or trazodone. The ratios of selectivity (NA/5-HT) for MCI-225, maprotiline, desipramine, imipramine and trazodone in the hypothalamic synaptosome were 14, 400, 300, 2.5 and 0.06, respectively. All compounds inhibited the uptake of [$^3$H]DA less potently than [$^3$H]NA or [$^3$H]5-HT.

### 3.2. Inhibition of radioligand binding

Table 3 shows that MCI-225 has a potent affinity for the 5-$HT_3$ receptor and NA transporter; the $IC_{50}$ values for the inhibition of [$^3$H]GR65630 and [$^3$H]nisoxetine binding were 81 and 53 nmol/l, respectively. The affinity for 5-HT transporter ($IC_{50}$ = 360 nmol/l) was lower than for NA transporter. The $K_i$ values for the NA and 5-HT transporter were 26 and 240 nmol/l, respectively. The ratio of selectivity (NA/5-HT) was 9.2. For the other binding site, MCI-225 affinity was remarkably lower. Fig. 2 shows that MCI-225 and ondansetron inhibit the binding of $^3$H-GR65630 for the 5-$HT_3$ receptor in N1E-115 cells. $K_i$ values for MCI-225, maprotiline, desipramine, imipramine, trazodone, and ondansetron were 3.04 nmol/l, 0.765 μmol/l, 1.98 μmol/l, 1.18 μmol/l, 30.9 μmol/l, and 1.68 nmol/l, respectively.

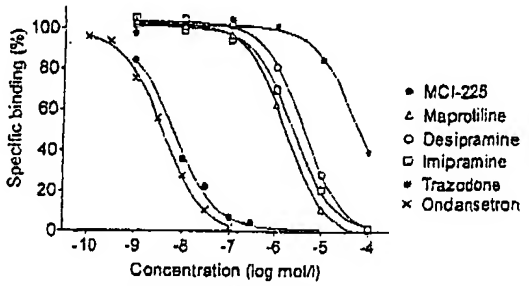


*Fig. 2:* Inhibition of [$^3$H]GR65630 binding to N1E-115 cells by MCI-225, maprotiline, desipramine, imipramine, trazodone and ondansetron. Each point shows the result of an experiment performed in duplicate.

### 3.3. Reserpine-induced hypothermia

Reserpine (5 mg/kg, i.p.) decreased the rectal temperature by approximately 9 °C 4 h after administration. As shown in Fig. 3, MCI-225 (0.3–10 mg/kg) dose-dependently reduced reserpine-induced hypothermia. Maprotiline (1 mg/kg), desipramine (0.3–10 mg/kg), and imipramine (1–10 mg/kg) also inhibited hypothermia. On the other hand, trazodone (0.1–3 mg/kg) tended to enhance hypothermia.

### 3.4. Tetrabenazine-induced ptosis

As shown in Fig. 4, tetrabenazine (12.5 mg/kg, i.p.) induced ptosis from 0.5 to 4 h after administration. MCI-225 (25 and 50 mg/kg) significantly inhibited the tetrabenazine-induced ptosis. The inhibition by MCI-225 (50 mg/kg) continued for 4 h after administration. Desipramine, imipramine and trazodone (25 and 50 mg/kg) also inhibited ptosis, although the effect of trazodone was slight. On the other hand, maprotiline did not significantly inhibit ptosis.

### 3.5. Yohimbine-induced lethality

Of 8 control mice treated with yohimbine (30 mg/kg), only 1 or 2 mice died. As shown in Table 4, MCI-225 remarkably enhanced yohimbine-induced lethality ($LD_{50}$ = 0.26 mg/kg). Maprotiline, desipramine and imipramine also potentiated the action of yohimbine ($LD_{50}$ = 4.37, 0.33, and 2.58 mg/kg, respectively). Trazodone showed no effect, even at a dose of 100 mg/kg.

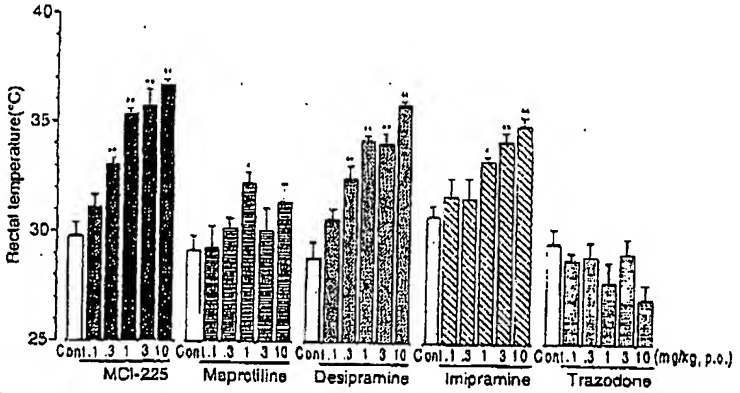


*Fig. 3:* Effects of MCI-225, maprotiline, desipramine, imipramine and trazodone on reserpine-induced hypothermia in mice. Rectal temperature was measured 4 h after the administration of test compounds (p.o.) and reserpine (5 mg/kg, i.p.). Data are presented as mean ± S.E. ($n = 6$). * $p < 0.05$, ** $p < 0.01$ compared to control group (Dunnett's two-tailed test following ANOVA).

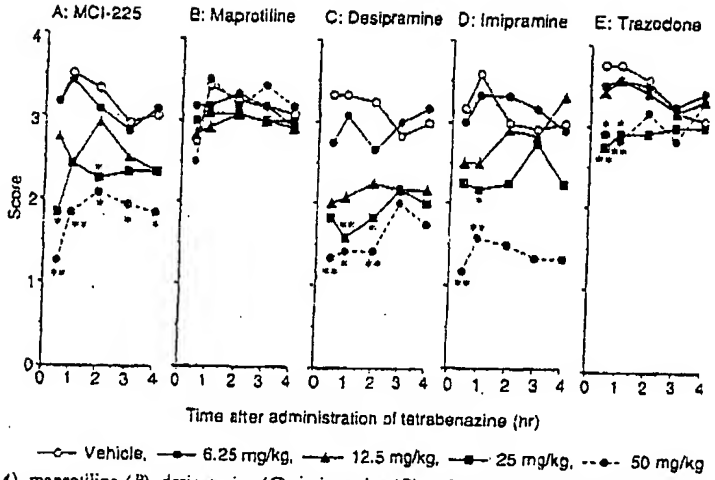


*Fig. 4:* Effects of MCI-225 (*A*), maprotiline (*B*), desipramine (*C*), imipramine (*D*) and trazodone (*E*) on tetrabenazine-induced ptosis in rats. Test compounds were administered orally 1 h before tetrabenazine (12.5 mg/kg, i.p.). Ptosis was scored from 0.5 to 4 h after the administration of test compounds (0 = normal, 1 = ¼, 2 = ½, 3 = ¾, 4 = complete closure of the eyelid). Data are presented as mean ($n = 6$). * $p < 0.05$, ** $p < 0.01$ compared to control group (non-parametric Dunnett's test following Kruskal-Wallis test).

*Table 4:* Effect of MCI-225, maprotiline, desipramine, imipramine and trazodone on yohimbine toxicity in mice. Yohimbine was given i.p. at a dose of 30 mg/kg, 1 h after the oral administration of test compounds. Lethality is represented as the number of mice that died/total number of mice. $LD_{50}$ values were calculated by the probit method.

| Compound | Lethality | | | | | | | | $LD_{50}$ (95 % confidence limit) |
|---|---|---|---|---|---|---|---|---|---|
| | Control | 0.1[a)] | 0.3[a)] | 1[a)] | 3[a)] | 10[a)] | 30[a)] | 100[a)] | |
| MCI-225 | 2/8 | 3/8 | 4/8 | 5/8 | 8/8** | 8/8** | 8/8** | 8/8** | 0.26 (0.06–0.57) |
| Maprotiline | 1/8 | | | 2/8 | 4/8 | 5/8 | 6/8* | 4/8 | 4.37 (0.003–60.26) |
| Desipramine | 2/8 | 3/8 | 3/8 | 7/8* | 5/8 | 7/8* | 8/8** | 8/8** | 0.33 (0.04–0.92) |
| Imipramine | 2/8 | | | 3/8 | 4/8 | 5/8 | 8/8** | 8/8** | 2.58 (0.55–5.77) |
| Trazodone | 2/8 | | | 3/8 | 2/8 | 1/8 | 2/8 | 3/8 | > 100 – |

[a)] Dose (mg/kg, p.o.)
* $p < 0.05$, ** $p < 0.01$, compared to control group (Fisher's exact probability test).

*Table 5:* Effects of MCI-225, maprotiline, desipramine, imipramine and trazodone on 5-HTP-induced head movements in rats. Test compounds were given orally 1 h before 5-HTP (150 mg/kg, i.p.) administration. The number of head movements was counted for 5 min 30 min after 5-HTP administration. Data are presented as mean ± S.E.

| Compound | n | Dose (mg/kg, p.o.) | | | |
|---|---|---|---|---|---|
| | | Control | 10 | 30 | 100 |
| MCI-225 | 8 | 1.4 ± 0.4 | 2.2 ± 0.4 | 1.8 ± 0.5 | 1.3 ± 0.6 |
| Maprotiline | 8 | 0.8 ± 0.4 | 1.1 ± 0.5 | 1.5 ± 0.7 | 2.3 ± 0.6 |
| Desipramine | 8 | 1.5 ± 0.4 | 1.6 ± 0.6 | 3.1 ± 0.4 | 1.1 ± 0.7 |
| Imipramine | 8 | 2.4 ± 0.7 | 2.5 ± 1.1 | 5.5 ± 3.4 | 13.1 ± 2.1** |
| Trazodone | 8 | 3.3 ± 1.0 | 1.0 ± 0.5 | 1.4 ± 0.5 | 1.3 ± 0.3 |

** $p < 0.01$, compared to control group (Dunnett's test following ANOVA).

### 3.6. 5-HTP-induced head movements

As shown in Table 5, 5-HTP (150 mg/kg, i.p.) induced head movements slightly. MCI-225, maprotiline, desipramine and trazodone (10–100 mg/kg) did not significantly change head movements in 5-HTP-treated rats. On the other hand, imipramine dose-dependently increased the number of head movements. At a dose of 100 mg/kg imipramine the enhancement was significant.

### 3.7. p-CA-induced hyperactivity

Table 6 shows that MCI-225 and desipramine (10–100 mg/kg) did not change p-CA-induced hyperactivity in rats, while both imipramine and trazodone produced dose-dependent reduction. The effects of imipramine

*Table 6:* Effects of MCI-225, maprotiline, desipramine, imipramine and trazodone on p-chloroamphetamine-induced hyperactivity in rats. p-Chloroamphetamine (5 mg/kg, s.c.) was given 30 min after the oral administration of test compounds. The spontaneous motor activity of two rats was measured for 90 min immediately after p-chloroamphetamine injection. Data are presented as mean ± S.E.

| Compound | n | Spontaneous motor activity (counts/90 min) | | | |
|---|---|---|---|---|---|
| | | Control | 10[a)] | 30[a)] | 100[a)] |
| MCI-225 | 6 | 7388 ± 823 | 6326 ± 841 | 7271 ± 697 | 6099 ± 1086 |
| Maprotiline | 6 | 7667 ± 746 | 6644 ± 629 | 7840 ± 744 | 4276 ± 841* |
| Desipramine | 6 | 8244 ± 677 | 7244 ± 240 | 5600 ± 1137 | 5667 ± 768 |
| Imipramine | 6 | 7147 ± 815 | 6690 ± 1045 | 5777 ± 1238 | 3100 ± 440* |
| Trazodone | 6 | 8825 ± 459 | 3298 ± 555** | 2562 ± 552** | 1368 ± 216** |

[a)] Dose (mg/kg, p.o.).
* $p < 0.05$, ** $p < 0.01$ compared to control group (Dunnett's two-tailed test following ANOVA).

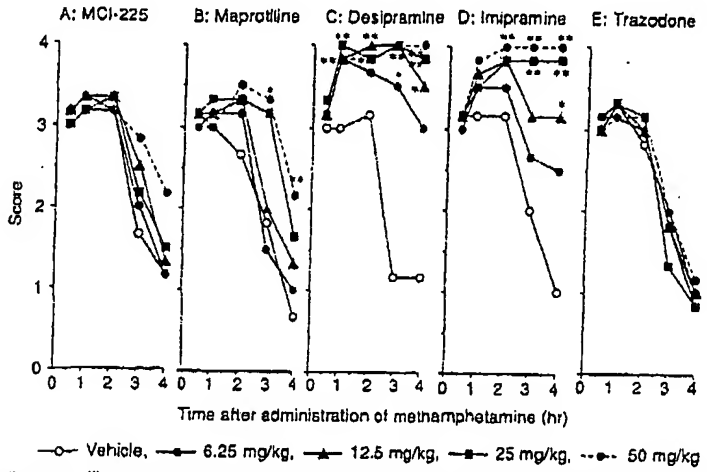


*Fig. 5:* Effects of MCI-225 (*A*), maprotiline (*B*), desipramine (*C*), imipramine (*D*), and trazodone (*E*) on methamphetamine-induced stereotypy in rats. Test compounds were administered orally 1 h before methamphetamine (3 mg/kg, s.c.) administration. Stereotypy was scored from 0.5 to 4 h after the administration of test compounds (0 = asleep, eyes closed, 1 = motionless, eyes open, 2 = moving around the cage, 3 = sniffing, 4 = continuous licking or gnawing). Data are presented as mean (n = 6). * $p < 0.05$, ** $p < 0.01$ compared to control group (non-parametric Dunnett's test following Kruskal-Wallis test.

(100 mg/kg) and trazodone (10–100 mg/kg) were significant. At a dose of 100 mg/kg maprotiline reduced the hyperactivity.

### 3.8. Methamphetamine-induced stereotyped behavior

Fig. 5 shows the score of stereotypy induced by methamphetamine (3 mg/kg, s.c.) in rat. MCI-225 did not significantly change the stereotypy at any doses tested, although 50 mg/kg of MCI-225 showed a tendency to enhance stereotypy. Maprotiline, desipramine and imipramine enhanced stereotypy at doses of 50, 6.25–50, and 12.5–50 mg/kg, respectively. Trazodone (6.25–50 mg/kg) did not show any effect.

### 3.9. L-DOPA-induced behavioral syndrome

Table 7 shows the score of L-DOPA (100 mg/kg, i.p.)-induced behaviors. MCI-225, maprotiline and imipramine (12.5–50 mg/kg) tended to enhance L-DOPA-induced behaviors, but only desipramine (50 mg/kg) significantly enhanced the behaviors. The effect of trazodone was not clear.

### 3.10. Forced swimming test

As shown in Fig. 6, the duration of immobility time in control rats was about 200–250 s. A single administration of MCI-225 (10–100 mg/kg) dose-dependently reduced the duration of immobility. With doses of 30 and 100 mg/kg, the decreases induced by MCI-225 were significant. Both imipramine and desipramine (100 mg/kg)

*Table 7:* Effects of MCI-225, maprotiline, desipramine, imipramine and trazodone on L-DOPA-induced behavior in mice. 19 h after the administration of nialamide (60 mg/kg, p.o.), mice were given test compounds orally. L-DOPA (100 mg/kg, i.p.) was administered 1.5 h later and L-DOPA-induced behaviors, mainly piloerection, salivation and excitation, and increased motor activity were scored according to the method of Ueki et al. [14] 1 h after the administration of L-DOPA (1 = slight, 2 = moderate with increased touch response, 3 = severe with increased touch response, biting and straub tail). Data are presented as mean.

| Compound | n | Dose (mg/kg, p.o.) | | | |
|---|---|---|---|---|---|
| | | Control | 12.5 | 25 | 50 |
| MCI-225 | 8 | 1.1 | 1.5 | 1.6 | 2.3 |
| Maprotiline | 8 | 1.1 | 1.9 | 1.8 | 2.1 |
| Desipramine | 8 | 1.4 | 1.8 | 2.0 | 2.6** |
| Imipramine | 8 | 1.1 | 1.3 | 1.8 | 2.0 |
| Trazodone | 8 | 1.4 | 1.3 | 1.6 | 1.8 |

** $p < 0.01$, compared to control group (non-parametric Dunnett's test following Kruskal-Wallis test).

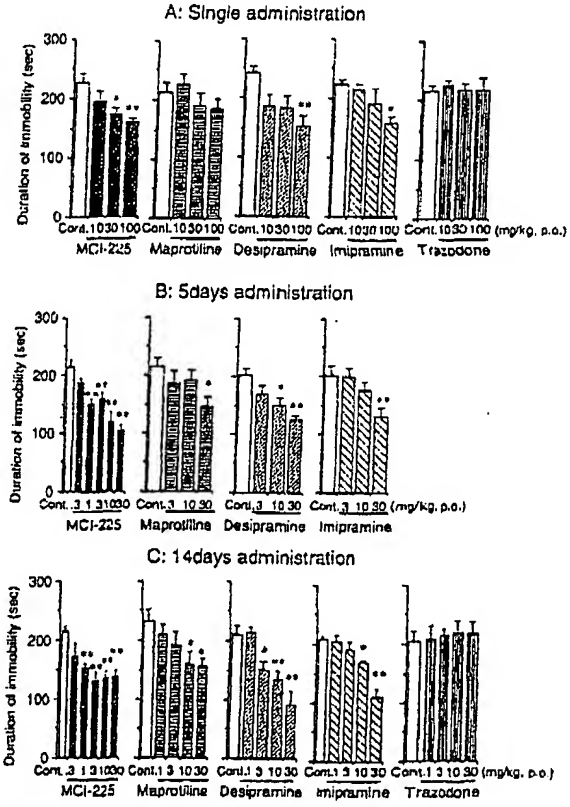


*Fig. 6:* Effects of MCI-225, maprotiline, desipramine, imipramine and trazodone on the duration of immobility in the forced swimming test in rats. Compounds were administered for 1 (*A*), 5 (*B*), or 14 (*C*) consecutive days. Rats were given 15 and 5 min forced swimming trials with a 24-h intertrial interval. The duration of immobility was recorded at the second trial. The last two administrations of the test compounds were done 1 h before the start of each forced swimming trial. Data are presented as mean ± S.E. (n = 6). * $p < 0.05$, ** $p < 0.01$ compared to control group (Dunnett's two-tailed test following ANOVA).

*Table 8:* Effects of a single administration of MCI-225, maprotiline, desipramine, imipramine and trazodone on spontaneous motor activity in rats. Test compounds were given orally 1 h before measurement. Data are presented as mean ± S.E.

| Compound | n | Spontaneous motor activity (counts/5 min) | | | |
|---|---|---|---|---|---|
| | | Control | 10[a)] | 30[a)] | 100[a)] |
| MCI-225 | 6 | 195 ± 27 | 159 ± 26 | 150 ± 11 | 124 ± 27 |
| Maprotiline | 6 | 180 ± 52 | 151 ± 12 | 170 ± 35 | 158 ± 17 |
| Desipramine | 6 | 206 ± 35 | 168 ± 20 | 103 ± 19* | 116 ± 10* |
| Imipramine | 6 | 180 ± 33 | 125 ± 14 | 152 ± 34 | 116 ± 15 |
| Trazodone | 6 | 150 ± 22 | 195 ± 22 | 194 ± 21 | 172 ± 12 |

[a)] Dose (mg/kg, p.o.).
* $p < 0.05$ compared to control group (Dunnett's two-tailed test following ANOVA).

also decreased the duration, and maprotiline (30 and 100 mg/kg) produced a slight decrease in the duration that was not significant. For both 5 and 14 days of administration the minimum effective dose of MCI-225 was 1 mg/kg. The minimum effective doses of maprotiline, desipramine and imipramine were 30, 10, and 30 mg/kg for 5 days administration, which decreased to 10, 3, and 10 mg/kg for 14 days administration, respectively. On the other hand, trazodone had no effect with either a single and 14-days administration.

### 3.11. Spontaneous motor activity

Table 8 shows that a single administration of MCI-225, maprotiline, imipramine or trazodone (10–100 mg/kg) did not change spontaneous motor activity in rats. Desipramine (30 and 100 mg/kg) decreased spontaneous motor activity. Administration of MCI-225 for 14 days (0.3–30 mg/kg) produced no change in the spontaneous motor activity of rats (data not shown).

### 3.12. Rapid eye movement sleep

As shown in Table 9, MCI-225 (10 mg/kg) decreased the REMS period without any change in SWS or wakefulness 6 h observation after administration. MCI-225 tended to increase REMS and SWS latencies. Desipramine and imipramine (10 mg/kg) decreased the REMS period with a significant increase in REMS latency. Trazodone (10 mg/kg) significantly increased REMS latency, while the effect of maprotiline (10 mg/kg) on both REMS period and REMS latency was not significant.

### 3.13. Von Bezold-Jarisch reflex

Fig. 7 shows that both MCI-225 and ondansetron reduced the ability of 5-HT (20 μg/kg, i.v.) to evoke the

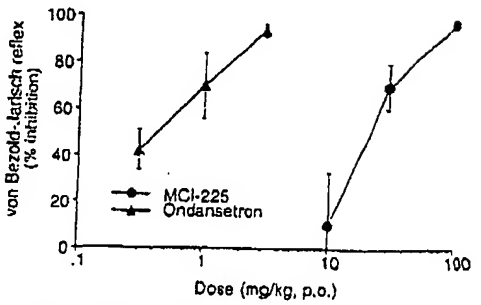


*Fig. 7:* Effects of MCI-225 and ondansetron on the von Bezold-Jarisch reflex in rats. Test compounds were administered orally 1 h before urethane (12.5 g/kg, i.p.). Under anesthesia, the right femoral vein was cannulated with a polyethylene tube, and ECG and heart rate were recorded with a polygraph. 5-HT (20 μg/kg) was injected via the cannula 1 h after urethane administration. 5-HT-induced bradycardia represented by the area under the curve was measured. Data are presented as mean ± S.E. ($n = 4$).

von Bezold-Jarisch reflex. The approximate $ID_{50}$ for MCI-225 and ondansetron were 22.2 and 0.29 mg/kg, p.o., respectively.

### 3.14. Oxotremorine-induced tremor, salivation and lacrimation

Fig. 8 and Table 10 shows that MCI-225 (3–100 mg/kg) did not inhibit oxotremorine (0.2 mg/kg, i.p.)-induced tremor, salivation, or lacrimation, while imipramine significantly inhibited tremor at doses of 30 and 100 mg/kg and lacrimation at 100 mg/kg. Imipramine (10–100 mg/kg) also inhibited salivation, but not significantly. High doses of desipramine and maprotiline tended to inhibit oxotremorine-induced changes, while trazodone showed no effect.

## 4. Discussion

In this study the neuropharmacological profile of MCI-225 and its antidepressant activity were examined using test procedures widely used. First, MCI-225 exhibited the profile of a potent NA uptake inhibitor. In vitro, MCI-225 inhibits NA uptake as much as maprotiline or imipramine, although less than desipramine. In vivo, MCI-225 inhibited reserpine-induced hypothermia and potentiated yohimbine-induced lethality as much as desipramine and more than maprotiline, imipramine or trazodone. In reserpine-induced hypothermia, which is one of the most frequently used tests to detect antidepressant action, many antidepressants are reported to antagonize

*Table 9:* Effects of MCI-225, maprotiline, desipramine, imipramine and trazodone on REMS, SWS and Wakefulness in rats. After 3-days habituation period to the experimental condition, ECoGs, EMG, and EOG of rats were recorded for 6 h after the oral administration of test compounds. Data are presented as mean ± S.E.

| Compound | Dose (mg/kg, p.o.) | n | REMS latency (min) | SWS latency (min) | REMS (%) | SWS (%) | Wakefulness (%) |
|---|---|---|---|---|---|---|---|
| MCI-225 | Control | 5 | 34.4 ± 7.2 | 9.3 ± 2.2 | 10.4 ± 0.8 | 70.0 ± 2.5 | 19.6 ± 2.1 |
| | 3 | 5 | 35.4 ± 6.3 | 13.0 ± 3.2 | 8.3 ± 1.3 | 67.3 ± 2.0 | 24.3 ± 2.4 |
| | 10 | 5 | 64.5 ± 24.5 | 16.4 ± 3.4 | 4.1 ± 0.5** | 67.7 ± 2.4 | 28.2 ± 2.7 |
| Maprotiline | Control | 5 | 37.4 ± 3.9 | 14.4 ± 3.2 | 10.6 ± 0.9 | 64.8 ± 0.8 | 24.6 ± 1.1 |
| | 10 | 5 | 47.0 ± 6.6 | 16.2 ± 4.3 | 8.9 ± 1.1 | 65.0 ± 1.5 | 26.1 ± 0.9 |
| Desipramine | Control | 5 | 24.2 ± 4.6 | 14.4 ± 3.7 | 8.9 ± 1.7 | 66.0 ± 1.6 | 25.1 ± 2.5 |
| | 3 | 5 | 45.8 ± 8.4 | 20.4 ± 1.8 | 5.9 ± 1.2 | 66.2 ± 2.0 | 27.8 ± 2.5 |
| | 10 | 5 | 95.0 ± 27.0* | 10.2 ± 4.0 | 0.7 ± 0.3** | 66.3 ± 1.3 | 33.0 ± 1.2** |
| Imipramine | Control | 5 | 39.6 ± 8.7 | 21.0 ± 7.1 | 8.4 ± 0.7 | 61.3 ± 1.8 | 30.3 ± 2.5 |
| | 3 | 5 | 44.1 ± 5.2 | 19.0 ± 4.4 | 10.2 ± 1.2 | 63.2 ± 2.0 | 26.7 ± 2.7 |
| | 10 | 5 | 177.2 ± 51.7* | 19.2 ± 4.7 | 1.2 ± 0.2** | 69.3 ± 3.5 | 29.6 ± 3.5 |
| Trazodone | Control | 5 | 31.4 ± 3.1 | 18.3 ± 1.5 | 11.3 ± 1.0 | 62.9 ± 2.5 | 25.8 ± 2.7 |
| | 3 | 5 | 46.7 ± 6.5 | 16.8 ± 3.5 | 9.8 ± 1.2 | 68.8 ± 1.5 | 21.3 ± 2.2 |
| | 10 | 5 | 63.4 ± 5.0** | 32.8 ± 7.6 | 7.3 ± 1.9 | 66.5 ± 1.7 | 26.2 ± 1.2 |

* $p < 0.05$, ** $p < 0.01$. compared to control group (Dunnett's test following ANOVA).

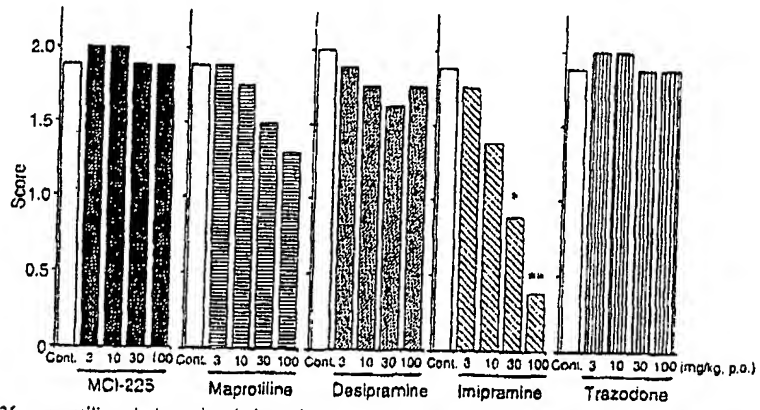


*Fig. 8:* Effects of MCI-225, maprotiline, desipramine, imipramine and trazodone on oxotremorine-induced tremor in mice. Test compounds were administered orally 1 h before the injection of oxotremorine (0.2 mg/kg, i.p.). After 30 min, tremor was scored (0 = no tremor, 1 = moderate, discontinuous tremor, 2 = intensive continuous tremor involving the whole body). Data are presented as mean (n = 8). * $p < 0.05$, ** $p < 0.01$ compared to control group (non-parametric Dunnett's test following Kruskal-Wallis test).

*Table 10:* Effects of MCI-225, maprotiline, desipramine, imipramine and trazodone on lacrimation and salivation induced by oxotremorine in mice. Oxotrmorine (0.2 mg/kg, i.p.) was injected 1 h after the oral administration of test compounds. Oxotremorine-induced lacrimation and salivation were observed 30 min after the injection of oxotremorine. Data are presented as the number of mice showing a positive response per number of mice tested.

| Compound | Dose (mg/kg, p.o.) | No. of mice positive/No. of mice tested | |
|---|---|---|---|
| | | Lacrimation | Salivation |
| MCI-225 | Control | 8/8 | 8/8 |
| | 3 | 8/8 | 8/8 |
| | 10 | 8/8 | 8/8 |
| | 30 | 8/8 | 8/8 |
| | 100 | 8/8 | 8/8 |
| Maprotiline | Control | 8/8 | 8/8 |
| | 3 | 8/8 | 8/8 |
| | 10 | 8/8 | 7/8 |
| | 30 | 7/8 | 8/8 |
| | 100 | 7/8 | 5/8 |
| Desipramine | Control | 8/8 | 8/8 |
| | 3 | 8/8 | 8/8 |
| | 10 | 8/8 | 8/8 |
| | 30 | 8/8 | 8/8 |
| | 100 | 6/8 | 7/8 |
| Imipramine | Control | 8/8 | 8/8 |
| | 3 | 7/8 | 7/8 |
| | 10 | 7/8 | 5/8 |
| | 30 | 6/8 | 4/8 |
| | 100 | 3/8* | 5/8 |
| Trazodone | Control | 8/8 | 8/8 |
| | 3 | 8/8 | 8/8 |
| | 10 | 8/8 | 8/8 |
| | 30 | 8/8 | 8/8 |
| | 100 | 8/8 | 8/8 |

* $p < 0.05$, compared to control group (Fisher's exact probability test).

the hypothermia [18]. This effect is of a central origin and depends on the activation of the noradrenergic mechanism resulting from the inhibition of noradrenaline uptake [19]. The potentiation of yohimbine-induced lethality is also used to predict antidepressant activity [18], which is exerted mainly through NA uptake inhibition and, to a lesser degree, in 5-HT uptake inhibition [20]. Therefore, the effects of MCI-225 in these tests suggest that MCI-225 inhibits NA uptake not only in vitro but also in vivo. In this study, maprotiline showed strong NA uptake inhibition in vitro, but its effect in vivo were less potent than MCI-225 or desipramine. Althouth the reason for this discrepancy is not clear, Balsara et al. [21] reported that maprotiline was significantly less potent than desipramine or imipramine in reserpine-induced ptosis in rats. Therefore, some experimental conditions in vivo may affect the potency of maprotiline. On the other hand, trazodone enhanced reserpine-induced hypothermia. This result is in accordance with earlier observation in mice [22] and rats [23].

Second, the receptor binding studies show that MCI-225 has selective affinity for the 5-$HT_3$ receptor among all receptors tested. Since MCI-225 inhibited the von Bezold-Jarisch reflex, a transient reflex bradycardia and hypotensive effect caused by activation of the cardiac 5-$HT_3$ receptor [24] in rats, MCI-225 is thought to have an antagonistic action on the 5-$HT_3$ receptor. 5-$HT_3$ receptor antagonists are reported to enhance acetylcholine release induced by potassium in rat entorhinal cortex [25] and ameliorate amnesia induced by scopolamine or basal forebrain lesions [26, 27]. As described before, MCI-225 reduced amnesia in both scopolamine-treated rats and basal forebrain-lesioned rats [7, 9]. These results also suggest that MCI-225 acts as a 5-$HT_3$ receptor antagonist in vivo. Matsumoto et al. [28] used microdialysis to show that ondansetron decreases the 5-HT or fluoxetine-induced inhibition of NA release in rat brain, thus it seems possible that the 5-$HT_3$ receptor antagonist action of MCI-225 decreased the endogenous 5-HT-induced inhibition of NA release. In fact, the potency of MCI-225 in reserpine-induced hypothermia and yohimbine-induced lethality is similar to that of desipramine, although the $K_i$ value of NA uptake inhibition by MCI-225 was less than that of desipramine. While the differences in the pharmacokinetics of these two NA uptake inhibitors may also affect the potency in these in vivo tests, the 5-$HT_3$ receptor antagonist action of MCI-225 may contribute to its antidepressant action. In contrast to MCI-225, other antidepressants tested showed no affinity for the 5-$HT_3$ receptor in N1E-115 cells. Thus the 5-$HT_3$ receptor antagonist activity of MCI-225 is thought to be a unique pharmacologic profile compared with these antidepressants.

Compared with NA uptake inhibition, the inhibition of 5-HT by MCI-225 was less potent both in vitro and in vivo. Present results in synaptosomal [$^3$H]monoamine uptake tests showed that both desipramine and maprotiline selectively inhibited NA uptake and trazodone selectively inhibited 5-HT uptake, while imipramine showed no selectivity. These results are similar to those reported earlier [29, 30]. The ratio of selectivity of uptake (NA / 5-HT) inhibition in the hypothalamus for MCI-225 was 14, while that for imipramine was 2.5. Furthermore, MCI-225 showed a more selective affinity for the NA transporter than the 5-HT transporter (ratio of selectiv-

ity = 9.2). This result correlates with that of the synaptosomal uptake and indicated that MCI-225 is a selective NA uptake inhibitor. In vivo, even at a dose of 100 mg/kg, p.o., MCI-225 did not change either 5-HTP-induced head movements or p-CA-induced hyperactivity, both of which are significantly affected by imipramine, similar to earlier observations [14, 13]. While trazodone inhibited 5-HT uptake in vitro, it did not potentiate 5-HTP-induced head movement. Since trazodone has been reported to have 5-HT receptor blocking action, this action may mask the effect induced by 5-HT uptake inhibition [23]. Trazodone has also been reported to inhibit 5-HTP-induced head twitch in mice [31]. In tetrabenazine-induced ptosis test, which is also frequently used to detect antidepressant action, MCI-225 inhibited tetrabenazine-induced ptosis as potent as both desipramine and imipramine. Bourin [18] showed that not only NA uptake inhibitors but also 5-HT uptake inhibitors antagonized this ptosis, so the potent 5-HT uptake inhibition by imipramine may contribute its action in this test. Both desipramine and imipramine showed the enhancements in the methamphetamine-induced stereotypy test, but MCI-225 had no significant effects. Since 5-$HT_3$ receptor antagonists decreased the release of DA in rat striatum in vivo [32] and inhibited the hyperactivity induced by infusion of dopamine into the ventral striatum of marmosets [33], the 5-$HT_3$ receptor antagonist action of MCI-225 may mask the enhancement induced by catecholamine uptake inhibition in this model. MCI-225 did not significantly change L-DOPA-induced behavior even at a dose of 100 mg/kg, p.o. MCI-225 also showed no significant effect on DA uptake in vitro, with a $K_i$ value 21000 times less potent than that for NA uptake. MCI-225 did not inhibit either MAO-A or MAO-B activity (data not shown), therefore neither DA uptake inhibition nor MAO-inhibition is thought to contribute to the antidepressant action of MCI-225.

The forced swimming test in rats shows a good predictive value for antidepressant potency in man [34]. In this test, MCI-225 reduced the immobility time after 1, 5, and 14 days of administration at lower doses than the other antidepressants tested. Since NA uptake inhibitors showed strong action in this test [16], NA uptake inhibition by MCI-225 is thought to contribute to its action. Furthermore, since 5-$HT_3$ receptor antagonists are also reported to reduce immobility [35], it may be possible that 5-$HT_3$ receptor antagonist action itself contributes to the reduction immobility by MCI-225. Since MCI-225 did not change spontaneous motor activity, its effect is not thought to be a false positive caused by effects on motor function. MCI-225 showed full activity with only 5-days administration, and 1 mg/kg, p.o. of MCI-225 significantly reduced the immobility time, while the effects of maprotiline, desipramine, and imipramine continued to increase after 14-days administration. Oral administration of imipramine has been reported to yield a more potent effect with 1 month treatment rather than 2 weeks treatment in this test [36]. A significant reduction in immobility by desipramine was observed on days 6–12 of treatment but not on days 1–5 [37]. These results suggest that MCI-225 will exhibit its full antidepressant activity with a short lag period in clinical trials. To confirm this hypothesis, examination of the clinical efficacy of MCI-225 in depressive patients will be needed. In this test, trazodone had no effect. This result is similar to that reported by Riblet et al. [38].

Even at a dose of 100 mg/kg, p.o. MCI-225 and trazodone did not inhibit oxotremorine-induced tremor, salivation or lacrimation, while imipramine inhibited and maprotiline and desipramine tended to inhibit at high doses. These results suggest that MCI-225 and trazodone do not exhibit central and peripheral anti-cholinergic effects. The lack of anti-cholinergic action of MCI-225 is thought to be due to its low affinity for muscarinic receptors and its 5-$HT_3$ receptor antagonist action. Coupled with the low affinity of MCI-225 for $H_1$ and $\alpha_1$ receptors, MCI-225 should not cause adverse effects mediated by the above receptors. In this study MCI-225 was found to reduce the REMS period and to show the tendency to increase REMS latency. But the suppression of REMS by MCI-225 was less potent than that by desipramine or imipramine. In addition to NA and 5-HT, cholinergic mechanisms have been implicated in REMS, and atropine, an anticholinergic agent, has been reported to suppress REMS [39]. Thus the weaker suppression of REMS by MCI-225 may reflect the lack of an anti-cholinergic effect. Another possibility is that the 5-$HT_3$ receptor antagonist action of MCI-225 may also contribute to its weakness in REMS suppression, since there has been a report to show that 5-$HT_3$ receptor antagonist increased REMS [40]. Periods of SWS and wakefulness were not changed by MCI-225 at doses that suppressed REMS. These results suggest that MCI-225 does not disturb sleep.

In conclusion, the present study reveals that MCI-225 is a selective NA uptake inhibitor with 5-$HT_3$ receptor antagonist action and that it has antidepressant activity as potent as TCAs in animal models. The 5-$HT_3$ receptor antagonist action of MCI-225 may contribute to its antidepressant action and reduce its anticholinergic action. MCI-225 appears to be a promising candidate for a new type of antidepressant.

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# Influence of Food on the Pharmacokinetics of a New Multiple Unit Sustained Release Sodium Valproate Formulation

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## Summary

The influence of concomitant food intake on the pharmacokinetics of sodium valproate (CAS 1039-66-5) was studied in 16 healthy male volunteers. A single dose of a new sustained release formulation containing 300 mg sodium valproate (Orfiril® long) was administered on two occasions either after a 12-h overnight fast or immediately after a standardised high energy high fat breakfast. A wash-out period of at least 1 week elapsed between the administrations. Valproate serum concentrations were measured by gas chromatography at intervals suitable for obtaining concentration-time curves for both regimens up to 72 h. The mean maximum serum concentration after fasting (17.0 µg/ml) was virtually the same as after a meal (16.8 µg/ml). Maximum concentrations were reached after 8 h for both nutritional states. The rate of elimination was not affected (terminal half-life approximately 15 h). The mean $AUC_{0-\infty}$ values were 468 µg/mlxh in fasting subjects and 458 µg/mlxh in postprandial subjects. The 90 % confidence intervals for all pharmacokinetic target parameters were entirely confined in the bioequivalence range of 80 to 125 %. The confidence intervals were even tighter, thus demonstrating homogeneity of drug release from the newly developed sodium valproate sustained release preparation. Bioequivalence with respect to extent and rate of absorption is therefore concluded for the comparison of fasting and non-fasting administration. The bioavailability of the sustained release sodium valproate preparation is not altered by the concomitant ingestion of food.